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(71) Applicant: CALGENE, INC. [US/US]; 1920 Fifth Street, Davis, CA 95616 (US).

(72) Inventors: HOUCK, Catherine, M. ; 172 Carmel Court, Vacaville, CA 95688 (US). PEAR, Julie, R. ; 710 Arthur Street, Davis, CA 95616 (US).

(74) Agent: RAE-VENTER, Barbara; Leydig, Voit & Mayer, 350 Cambridge Avenue, Suite 200, Palo Alto, CA 94306 (US).

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(54) Title: FRUIT-SPECIFIC TRANSCRIPTIONAL FACTORS

(57) Abstract

Fruit-specific regulatory regions are identified employing cDNA screening. The resulting fruit-specific regulatory regions are manipulated for use with foreign sequences for introduction into plant cells to provide transformed plants having fruit with a modified phenotypic property. The invention is exemplified with a tomato fruit-specific promoter which is active throughout the stages of fruit ripening.

FRUIT-SPECIFIC TRANSCRIPTIONAL FACTORS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of Application Serial No. 168,190, filed March 15, 1988, which is a continuation-in-part of Application Serial No. 054,369 filed May 26, 1987, which applications are incorporated herein by reference.

INTRODUCTION

Technical Field

This invention relates to DNA expression cassettes capable of directing fruit-specific expression of *in vitro* constructed expression cassettes in plants. The invention is exemplified by promoters useful in fruit-specific transcription in a tomato plant.

Background

Manipulation of plants has proven to be significantly more difficult than manipulation of prokaryotes and mammalian hosts. As compared to prokaryotes and mammalian cells, much less was known about the biochemistry and cell biology of plant cells and plants. The ability to transform plant cells and regenerate plants is unique to flora since other differentiated species provide readily available transformable germ cells which may be fertilized and introduced into the live host for fetal development to a mature fetus. There has been substantial interest in modifying the ovum with inducible transcriptonal initiation regions to afford inducible transcription and expression of the gene introduced into the ovum, rather than constituitive expression which would result in expression throughout the fetus.

Also, for plants, it is frequently desirable to be able to control expression at a particular stage in the growth of the plant or in a particular plant part. During the various stages of the growth of the plant, and as to the various components of the plant, it will frequently be desirable to direct the effect of the construct introduced into the entire plant or a particular part and/or to a particular stage of differentiation of the plant cell. For this purpose, regulatory sequences are required which afford the desired initiation of transcription in the appropriate cell types and/or at the appropriate time in the plant development, without having serious detrimental effects on the plant development and productivity.

It is therefore important to be able to isolate sequences which can be manipulated to provide the desired regulation of transcription in a plant cell host during the growing cycle of the plant. One aspect of this interest is the ability to change the phenotype of fruit, so as to provide fruit which will have improved aspects for storage, handling, cooking, organoleptic properties, freezing, nutritional value, and the like.

Relevant Literature

cDNA clones from tomato displaying differential expression during fruit development have been isolated and characterized (Mansson et al., Mol. Gen. Genet. (1985) 200:356-361; Slater et al., Plant Mol. Biol. (1985) 5:137-147). The studies have focused primarily on mRNAs which accumulate during fruit ripening. One of the proteins encoded by the ripening-specific cDNAs has been identified as polygalacturonase (Slater et al., Plant Mol. Biol. (1985) 5:137-147). A cDNA clone which encodes tomato polygalacturonase has been sequenced. Grierson et al., Nucleic Acids Research (1986) 14:8395-8603. The concentration of polygalac-

turonase mRNA increases 2000-fold between the immature-green and red-ripe stages of fruit development. This suggests that expression of the enzyme is regulated by the specific mRNA concentration which in turn is regulated by an increase in transcription. Della Penna et al., Proc. Natl. Acad. Sci. USA (1986) 83:6420-6424. Mature plastid mRNA for psbA (one of the components of photosystem II) reaches its highest level late in fruit development, whereas after the onset of ripening, plastid mRNAs for other components of photosystem I and II decline to nondetectable levels in chromoplasts. Piechulla et al., Plant Mol. Biol. (1986) 7:367-376.

Other studies have focused on cDNAs encoding genes under inducible regulation, e.g. proteinase inhibitors which are expressed in response to wounding in tomato (Graham et al., J. Biol. Chem. (1985) 260:6555-6560; Graham et al., J. Biol. Chem. (1985) 260:6561-6564) and on mRNAs correlated with ethylene synthesis in ripening fruit and leaves after wounding. Smith et al., Planta (1986) 168:94-100.

Leaf disc transformation of cultivated tomato is described by McCormick, et al., Plant Cell Reports (1986) 5:81-89.

SUMMARY OF THE INVENTION

Novel DNA constructions are provided employing a "fruit-specific promoter," particularly those active beginning at or shortly after anthesis or beginning at the breaker stage, joined to a DNA sequence of interest and a transcriptional termination region. A DNA construct may be introduced into a plant cell host for integration into the genome and transcription regulated at a time at or subsequent to anthesis. In this manner, high levels of RNA and, as appropriate, polypeptides, may be achieved during formation and/or ripening of fruit.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the nucleotide sequence of the cDNA clones pCGN1299 (2A11) and pCGN1298 (3H11). The amino acid sequence of the polypeptide encoded by the open reading frame is also indicated.

Figure 2 is a comparison of 2A11 to pea storage proteins and other abundant storage proteins:

(a) 2A11 (residues 33-46) is compared to PA1b and the reactive site sequences of some protease inhibitors, PA1b (residues 6-23), chick pea inhibitor (residues 11-23), lima bean inhibitor (residues 23-35), human α1-antitrypsin reactive site peptide. The arrow indicates the reactive site.

(b) is a comparison of the amino terminal sequence of 2A11 with the amino termini of a range of seed proteins. The data have been modified or deletions introduced to maximize homology; conserved residues are shown boxed. The sequences are from the following sources: PA1b; barley chloroform/methanol-soluble protein d; wheat albumin; wheat α-amylase inhibitor 0.28; millet bi-functional inhibitor; castor bean 2S small subunit; and napin small subunit.

Figure 3 is a schematic diagram of the construction of the binary plasmid pCGN783; (a) through (f) refer to the plasmid constructions in Example 6.1.

Figure 4 shows the complete sequence of the 2A11 genomic DNA cloned into pCGN1273 from the XhoI site (position 1 at the 5' end) to the EcoRI site (position 4654).

Figure 5 shows the nucleotide sequence of a polygalacturonase (PG) genomic clone.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

In accordance with the subject invention, DNA constructs are provided which allow for modification of plant phenotype during fruit maturation and ripening. The DNA constructs provide for a regulated transcrip-

tional initiation region associated with fruit development and ripening. Downstream from and under the transcriptional initiation regulation of the fruit related initiation region will be a sequence of interest which will provide for modification of the phenotype of the fruit. Desirably, integration constructs may be prepared which allow for integration of the transcriptional cassette into the genome of a plant host. Conveniently, the vector may include a multiple cloning site downstream from the fruit related transcriptional initiation region, so that the integration construct may be employed for a variety of sequences in an efficient manner.

Of particular interest is a transcriptional initiation region which is activated at or shortly after anthesis, so that in the early development of the fruit, it provides the desired level of transcription of the sequence of interest. Normally, the sequence of interest will be involved in affecting the process in the early formation of the fruit or providing a property which is desirable during the growing (expansion) period of the fruit, or at or after harvesting.

The ripening stages of the tomato may be broken down into mature green, breaker, turning, pink, light red and red. Desirably, the transcriptional initiation region maintains its activity during the expansion and maturation of the green fruit, more desirably continues active through the ripening or red fruit period. Comparable periods for other fruit are referred to as stages of ripening. The invention is not limited to those transcriptional initiation regions which are activated at or shortly after anthesis but also includes transcriptional initiation regions which are activated at any of the ripening stages of the fruit.

The transcriptional initiation region may be native or homologous to the host or foreign or heterologous to the host. By foreign is intended that the transcriptional initiation region is not found in the wild-type host into which the transcriptional initiation region is introduced. Of particular interest is a tomato fruit-specific transcriptional initiation region referred to as 2A11 which regulates the expression of a 2A11 cDNA sequence described in the Experimental section. The 2A11 transcriptional initiation region provides for an abundant messenger, being activated at or shortly after anthesis and remaining active until the red fruit stage. The expressed protein is a sulfur-rich protein similar to other plant storage proteins in sulfur content and size. Also of interest is the transcriptional initiation region which regulates expression of the enzyme polygalacturonase, an enzyme which plays an important role in fruit ripening. The polygalacutonase promoter is active in at least the breaker through red fruit stage.

Other fruit-specific promoters may be activated at times subsequent to anthesis, such as prior to or during the green fruit stage, during pre-ripe (e.g., breaker) or even into the red fruit stage.

A transcriptional initiation region may be employed for varying the phenotype of the fruit. Various changes in phenotype are of interest. These changes may include up- or down-regulation of formation of a particular saccharide, involving mono- or polysaccharides, involving such enzymes as polygalacturonase, levansucrase, dextransucrase, invertase, etc.; enhanced lycopene biosynthesis; cytokinin and monellin synthesis. Other properties of interest for modification include response to stress, organisms, herbicides, bruising, mechanical agitation, etc., change in growth regulators, organoleptic properties, etc. For antisense or complementary sequence transcription, the sequence will

usually be at least 12, more usually at least 16 nt. Antisense sequences of interest include those of polygalacturonase, sucrase synthase and invertase.

The transcriptional cassette will include in the 5'-3' direction of transcription, a transcriptional and translational initiation region, a sequence of interest, and a transcriptional and translational termination region functional in plants. One or more introns may be also be present. The DNA sequence may have any open reading frame encoding a peptide of interest, e.g. an enzyme, or a sequence complementary to a genomic sequence, where the genomic sequence may be an open reading frame, an intron, a non-coding leader sequence, or any other sequence where the complementary sequence will inhibit transcription, messenger RNA processing, e.g. splicing, or translation. The DNA sequence of interest may be synthetic, naturally derived, or combinations thereof. Depending upon the nature of the DNA sequence of interest, it may be desirable to synthesize the sequence with plant preferred codons. The plant preferred codons may be determined from the codons of highest frequency in the proteins expressed in the largest amount in the particular plant species of interest.

In preparing the transcription cassette, the various DNA fragments may be manipulated, so as to provide for the DNA sequences in the proper orientation and, as appropriate, in the proper reading frame. Toward this end, adapters or linkers may be employed for joining the DNA fragments or other manipulations may be involved to provide for convenient restriction sites, removal of superfluous DNA, removal of restriction sites, or the like. Toward this end, <u>in vitro</u> mutagenesis, primer repair, restriction, annealing, resection, ligation, or the like may be employed, where insertions, deletions or substitutions, e.g. transitions and transversions, may be involved.

The termination region which is employed will be primarily one of convenience, since the termination regions appear to be relatively interchangeable. The termination region may be native with the transcriptional initiation region, may be native with the DNA sequence of interest, or may be derived from another source. Convenient termination regions are available from the Ti-plasmid of A. tumefaciens, such as the octopine synthase and nopaline synthase termination regions.

By appropriate manipulations, such as restriction, chewing back or filling in overhangs to provide blunt ends, ligation of linkers, or the like, complementary ends of the fragments can be provided for joining and ligation.

In carrying out the various steps, cloning is employed, so as to amplify the amount of DNA and to allow for analyzing the DNA to ensure that the operations have occurred in proper manner. A wide variety of cloning vectors are available, where the cloning vector includes a replication system functional in E. coli and a marker which allows for selection of the transformed cells. Illustrative vectors include pBR332, pUC series, M13mp series, pACYC184, etc. Thus, the sequence may be inserted into the vector at an appropriate restriction site(s), the resulting plasmid used to transform the E. coli host, the E. coli grown in an appropriate nutrient medium and the cells harvested and lysed and the plasmid recovered. Analysis may involve sequence analysis, restriction analysis, electrophoresis, or the like. After each manipulation the DNA sequence to be used in the final construct may be restricted and joined to the next sequence, where each of the partial constucts may be cloned in the same or different plasmids.

In addition to the transcription construct, depending upon the manner of introduction of the transcription construct into the plant, other DNA sequences may be required. For example, when using the Ti- or Ri-plasmid for transformation of plant cells, as described below, at least the right border and frequently both the right and left borders of the T-DNA of the Ti- or Ri-plasmids will be joined as flanking regions to the transcription construct. The use of T-DNA for transformation of plant cells has received extensive study and is amply described in EPA Serial No. 120,516, Hoekema, In: The Binary Plant Vector System Offset-drukkerij Kanters B.V., Alblasserdam, 1985, Chapter V, Knauf et al., Genetic Analysis of Host Range Expression by Agrobacterium, In:Molecular Genetics of the Bacteria-Plant Interaction, Puhler, A. ed.,Springer-Verlag, NY, 1983, p.245, and An et al., EMBO J. (1985) 4:277-284

Alternatively, to enhance integration into the plant genome, terminal repeats of transposons may be used as borders in conjunction with a transposase. In this situation, expression of the transposase should be inducible, so that once the transcription construct is integrated into the genome, it should be relatively stably integrated and avoid hopping.

The transcription construct will normally be joined to a marker for selection in plant cells. Conveniently, the marker may be resistance to a biocide, particularly an antibiotic, such as kanamycin, G418, bleomycin, hygromycin, chloramphenicol, or the like. The particular marker employed will be one which will allow for selection of transformed cells as compared to cells lacking the DNA which has been introduced.

A variety of techniques are available for the introduction of DNA into a plant cell host. These techniques include transformation with Ti-DNA employing A. tumefaciens or A. rhizogenes as the transforming agent, protoplast fusion, injection, electroporation,

etc. For transformation with Agrobacterium, plasmids can be prepared in E. coli which plasmids contain DNA homologous with the Ti-plasmid, particularly T-DNA. The plasmid may or may not be capable of replication in Agrobacterium, that is, it may or may not have a broad spectrum prokaryotic replication system, e.g., RK290, depending in part upon whether the transcription construct is to be integrated into the Ti-plasmid or be retained on an independent plasmid. By means of a helper plasmid, the transcription construct may be transferred to the A. tumefaciens and the resulting transformed organism used for transforming plant cells.

Conveniently, explants may be cultivated with the A. tumefaciens or A. rhizogenes to allow for transfer of the transcription construct to the plant cells, the plant cells dispersed in an appropriate selective medium for selection, grown to callus, shoots grown and plantlets regenerated from the callus by growing in rooting medium. The Agrobacterium host will contain a plasmid having the vir genes necessary for transfer of the T-DNA to the plant cells and may or may not have T-DNA. For injection and electroporation, disarmed Ti-plasmids (lacking the tumor genes, particularly the T-DNA region) may be introduced into the plant cell.

As a host cell, any of a number of fruit bearing plants may be employed in which the plant parts of interest are derived from the ovary wall. These include true berries such as tomato, grape, blueberry, cranberry, currant, and eggplant; stone fruits (drupes) such as cherry, plum, apricot, peach, nectarine and avocado; compound fruits (druplets) such as raspberry and blackberry. In hesperidium (oranges, citrus), the expression cassette might be expected to be expressed in the "juicy" portion of the fruit. In pepos (such as watermelon, cantelope, honeydew, cucumber and squash) the equivalent tissue for expression is most likely the inner edible portions, whereas in legumes (such as

peas, green beans, soybeans) the equivalent tissue is the seed pod.

Identifying useful transcriptional initiation regions may be achieved in a number of ways. Where a fruit protein has been or is isolated, it may be partially sequenced, so that a probe may be designed for identifying messenger RNA specific for fruit. To further enhance the concentration of the messenger RNA specifically associated with fruit, cDNA may be prepared and the cDNA subtracted with messenger RNA or cDNA from non-fruit associated cells. The residual cDNA may then be used for probing the genome for complementary sequences, using an appropriate library prepared from plant cells. Sequences which hybridize to the cDNA may then be isolated, manipulated, and the 5'-untranslated region associated with the coding region isolated and used in espression constructs to identify the transcriptional activity of the 5'-untranslated region. In some instances, a probe may be employed directly for screening a genomic library and identifying sequences which hybridize to the probe. The sequences will be manipulated as described above to identify the 5'-untranslated region.

As an example, a promoter of particular interest for the subject invention, the fruit-specific transcriptional initiation region (promoter) from a DNA sequence which encodes a protein described as 2A11 in the Experimental section was identified as follows. cDNA clones made from ripe fruit were screened using cDNA probes made from ripe fruit, green fruit, and leaf mRNA. Clones were selected having more intense hybridization with the fruit DNAs as contrasted with the leaf cDNAs. The screening was repeated to identify a particular cDNA referred to as 2A11. The 2A11 cDNA was then used for screening RNA from root, stem, leaf, and seven stages of fruit development after the mRNA was sized on gels. The screening demonstrated that the

particular message was present throughout the seven stages of fruit development. The mRNA complementary to the specific cDNA was absent in other tissues which were tested. The cDNA was then used for screening a genomic library and a fragment selected which hybridized to the subject cDNA. The 5' and 3' non-coding regions were isolated and manipulated for insertion of a foreign sequence to be transcribed under the regulation of the 2A11 promoter.

The cells which have been transformed may be grown into plants in accordance with conventional ways. See, for example, McCormick et al., Plant Cell Reports (1986) 5:81-84. These plants may then be grown, and either pollinated with the same transformed strain or different strains, identifying the resulting hybrid having the desired phenotypic characteristic. Two or more generations may be grown to ensure that the subject phenotypic characteristic is stably maintained and inherited and then seeds harvested for use to provide fruits with the new phenotypic property.

A protein is provided having the sequence described in the Experimental section designated as 2A11. This protein could be a storage protein and be useful in enhancing sulfur containing amino acids (cysteine and methionine) in the diet. It can be obtained in substantially pure form by providing for expression in prokaryotes or eukaryotes, e.g., yeast by inserting the open reading frame into an expression cassette containing a transcriptional initiation region. A variety of expression cassettes are commercially available or have been described in the literature. See, for example, U.S. Patent Nos. 4,532,207; 4,546,082; 4,551,433; and 4,559,302. The product, if intracellular, may be isolated by lysing of the cells and purification of the protein using electrophoresis, affinity chromatography, HPLC extraction, or the like. The product may be isolated in substantially pure form free of other plant

products, generally having at least about 95% purity, usually at least about 99% purity.

The following examples are offered by way of illustration and not by limitation.

EXPERIMENTAL

Example 1

Construction of Tomato Ripe Fruit cDNA Bank and Screening for Fruit-Specific Clones

Tomato plants (Lycopersicon esculentum cv UC82B) were grown under greenhouse conditions. Poly(A)$^+$RNA was isolated as described by Mansson et al., Mol. Gen. Genet. (1985) 200:356-361. The synthesis of cDNA from poly(A)$^+$ RNA prepared from ripe fruit, cloning into the PstI site of the plasmid pUC9 and transformation into an E. coli vector were all as described in Mansson et al., Mol. Gen. Genet. (1985) 200:356-361.

Library Screening

Two thousand recombinant clones were screened by colony hybridization with radiolabeled cDNA made from tomato red fruit mRNA, immature green fruit mRNA, and leaf mRNA. Bacterial colonies immobilized onto GeneScreen Plus filters (New England Nuclear), were denatured in 1.5 M NaCl in 0.5 M NaOH, then neutralized in 1.5 M NaCl in 0.5 M Tris-HCl pH 8, and allowed to air dry. Hybridization, washing and autoradiography were all performed as described in Maniatis et al., Molecular Cloning: A Laboratory Manual (1982) Cold Spring Harbor, New York.

Sixty-five clones were selected which had more intense hybridization signals with fruit cDNA than with leaf cDNA and therefore appeared to be under-represented in the leaf mRNA population relative to the fruit population. Replicate slot blot filters were prepared using purified DNA from the selected clones and hybrid-

ized with radioactive cDNA from leaf, green fruit, and red fruit as before. This allowed selection of cDNA clone 2A11, also referred to as pCGN1299 which is on at high levels in both the fruit stages (red and green) and off in the leaf.

Example 2

Analysis of Clones

Synthesis of RNA Probes

The cDNA insert of pCGN1299 was excised as an EcoRI to HindIII fragment of approximately 600 bp (as measured on an agarose gel), and subcloned into the Riboprobe vector pGEM1 (Promega Biotec), creating pCGN488. ^{32}P-labeled transcripts made from each strand of the pCGN488 insert using either SP6 or T7 polymerase were used as probes in separate Northern blots containing mRNA from leaf, immature green and mature red fruits. The RNA transcript from the SP6 promoter did not hybridize to the tomato mRNA. However, the transcript from the T7 promoter hybridized to an mRNA of approximately 700 nt in length from the green fruit and the red fruit but not to mRNA from tomato leaf. The direction of transcription of the corresponding mRNA was thus determined.

The tissue specificity of the pCGN1299 cDNA was demonstrated as follows. RNA from root, stem, leaf, and seven stages of fruit development (immature green, mature green, breaker, turning, pink, light red, and red) was sized on formaldehyde/agarose gels according to the method described by Maniatis et al., (1982), immobilized on nitrocellulose and hybridized to ^{32}P-labeled RNA which was synthesized in vitro from pCGN488 using T7 polymerase. Each lane contained 100 ng of polyA$^+$ RNA except for two lanes (pink and light red lanes) which contained 10 µg of total RNA. The Northern analysis of mRNA from root, stem, leaf, and various stages of fruit development indicated that

pCGN1299 cDNA was expressed in all stages of fruit development from the early stages immediately after anthesis to red ripe fruit. No mRNA hybridizing to pCGN1299 was found in leaf, stem, or root tissue. The size of the mRNA species hybridizing to the pCGN488 probe was approximately 700 nt.

Message abundance corresponding to the pCGN1299 cDNA was determined by comparing the hybridization intensity of a known amount of RNA synthesized in vitro from pCGN488 using SP6 polymerase to mRNA from red tomato fruit in a Northern blot. The ^{32}P-labeled transcript from pCGN488 synthesized in vitro using T7 polymerase was used as a probe. The Northern analysis was compared to standards which indicated that the pCGN1299 cDNA represents an abundant mRNA class in tomato fruit, being approximately 1% of the message.

Example 3
Sequencing of pCGN1299 and pCGN1298 cDNA Clones

DNA Sequencing

The polyA+ sequence was missing from pCGN1299 cDNA. A longer cDNA clone, pCGN1298, therefore was identified by its hybridization with the pCGN488 probe. The complete DNA sequence of the two cDNA inserts was determined using both Maxam-Gilbert and the Sanger dideoxy techniques and is as follows. The sequence of pCGN1298 contains additional sequences at both the 5' and 3' end compared to pCGN1299. As shown in Figure 1, the sequences are identical over the region that the two clones have in common.

Amino Acid Sequence

The pCGN1299 cDNA sequence was translated in three frames. The longest open reading frame (which starts from the first ATG) is indicated. Both pCGN1299 and pCGN1298 have an open reading frame which encodes a

96 amino acid polypeptide (see Figure 1). The protein has a hydrophobic N-terminus which may indicate a leader peptide for protein targeting. A hydrophobicity profile was calculated using the Hopp and Woods, (Proc. Natl. Acad. Sci. USA (1981) 78:3824-3828) algorithm. Residues 10-23 have an extremely hydrophobic region. A comparison of 2A11 to pea storage proteins and other abundant storage proteins is shown in Figure 2. The sulfur-rich composite of the fruit-specific protein is similar to a pea storage protein which has recently been described (see Higgins et al., J. Biol. Chem. (1986) 261:11124-11130, for references to the individual peptides). This may indicate a storage role for this fruit-specific protein abundant species.

Example 4
Screening Genomic Library for Genomic Clones

Southern Hybridization

Southern analysis was performed as described by Maniatis et al., 1982. Total tomato DNA from cultivar UC82B was digested with EcoRI or HindIII, separated by agarose gel electrophoresis and transferred to nitrocellulose. Southern hybridization was performed using a ^{32}P-labeled probe produced by nick translation of pCGN488 (Maniatis et al., 1982). The simple hybridization pattern indicated that the gene encoding pCGN1299 cDNA was present in a few or perhaps even one copy in a tomato genome.

Isolation of a Genomic Clone

A genomic library established in Charon35/Sau3A constructed from DNA of the tomato cultivar VFNT-Cherry was screened using the [^{32}P]-RNA from cDNA clone pCGN488 as a probe. A genomic clone containing approximately 12.5 kb of sequence from the tomato genome was isolated. The region which hybridizes to a pCGN488

probe spans an XbaI restriction site which was found in the cDNA sequence and includes the transcriptional initiation region designated 2A11.

Sequence of Genomic Clone

The DNA sequence of the genomic clone was determined by Sanger dideoxy techniques and is as shown in Figure 4. The sequence of the genomic clone is identical to the pCGN1299 cDNA clone over the region they have in common.

Subcloning

The region surrounding the XbaI restriction site, approximately 2.4 kb in the 5' direction and approximately 2.1 kb in the 3' direction was subcloned to provide an expression cassette. The 5' XhoI to XbaI fragment and the 3' XbaI to EcoRI fragment from the 2A11 genomic clone were inserted into a pUC-derived chloromphenicol plasmid containing a unique XhoI site and no XbaI site. This promoter cassette plasmid is called pCGN1273.

Example 5

Construction of Fruit-Specific Antisense Cassette

Insertion of Antisense Fragment

The 2A11 genomic fragment was tagged with PG antisense sequences by insertion of PG into the unique XbaI site of the pCGN1273 promoter cassette in the antisense orientation. The inserted sequences increased the size of the mRNA over the endogenous transcript, and thus the expression pattern of the construct could be compared to the endogenous gene by a single Northern hybridization in a manner analogous to the detection of a tuber-specific potato gene described by Eckes et al., Mol. Gen. Genet. 1986 205:14-22.

Example 6

Insertion of Tagged Genomic Construction Into Agrobacterium Binary Vectors

The tagged genomic construction is excised using the flanking XhoI restriction enzyme sites and is cloned into the unique SalI site of the binary plasmid pCGN783 containing a plant kanamycin resistance marker between the left and right borders to provide plasmid pCGN1269.

This plasmid binary vector in E. coli C2110 is conjugated into A. tumefaciens containing a disarmed Ti-plasmid capable of transferring the polygalacturonase antisense cassette and the kanamycin resistance cassette into the plant host genome.

The Agrobacterium system which is employed is A. tumefaciens PC2760 (G. Ooms et al., Plasmid (1982) 7:15-29; Hoekema et al., Nature (1983) 303:179-181; European Patent Application 84-200239.6, 2424183).

1. Construction of pCGN783

pCGN783 is a binary plasmid containing the left and right T-DNA borders of A. tumefaciens octopine Ti-plasmid pTiA6 (Currier and Nester, J. Bacteriol. (1976) 126:157-165) the gentamicin resistance gene of pPH1J1 (Hirsch et al., Plasmid (1984) 12:139-141), the 35S promoter of cauliflower mosaic virus (CaMV) (Gardner et al., Nucleic Acid Res. (1981) 9:1871-1880); the kanamycin resistance gene of Tn5 (Jorgensen, Mol. Gen. (1979) 177:65); and the 3' region from transcript 7 of pTiA6 (Currier and Nester, supra (1976)). A schematic diagram of the construction of pCGN783 is shown in Figure 3. (a) through (f) refer to the plasmid constructions detailed below.

(a) Construction of pCGN587

The HindIII-SmaI fragment of Tn5 containing the entire structural gene for APH3'II (Jorgensen et al., Mol. Gen. (1979) 177:65), was cloned into pUC8 (Vieira and Messing, Gene (1982) 19:259), converting the fragment into a HindIII-EcoRI fragment, since there is an EcoRI site immediately adjacent to the SmaI site. The PstI-EcoRI fragment containing the 3' portion of the APH3'II gene was then combined with an EcoRI-BamHI-SalI-PstI linker into the EcoRI site of pUC7 (pCGN546W). Since this construct does not confer kanamycin resistance, kanamycin resistance was obtained by inserting the BglI-PstI fragment of the APH3'II gene into the BamHI-PstI site (pCGN546X). This procedure reassembles the APH3'II gene, so that EcoRI sites flank the gene. An ATG codon was upstream from and out of reading frame with the ATG initiation codon of APH3'II. The undesired ATG was avoided by inserting a Sau3A-PstI fragment from the 5' end of APH3'II, which fragment lacks the superfluous ATG, into the BamHI-PstI site of pCGN546W to provide plasmid pCGN550. The EcoRI fragment of pCGN550 containing the APH3'II gene was then cloned into the EcoRI site of pUC8-pUC13 (K. Buckley supra (1985)) to give pCGN551.

Each of the EcoRI fragments containing the APH3'II gene was then cloned into the unique EcoRI site of pCGN451, which contains an octopine synthase cassette for expression to provide pCGN548 (2ATG)) and pCGN552 (1ATG). The plasmid pCGN451 having the ocs 5' and the ocs 3' in the proper orientation was digested with EcoRI and the EcoRI fragment from pCGN551 containing the intact kanamycin resistance gene inserted with EcoRI site to provide pCGN552 having the kanamycin resistance gene in the proper orientation. This ocs/KAN gene was used to provide a selectable marker for the trans type binary vector pCGN587.

The 5' portion of the engineered octopine synthase promoter cassette consists of pTiA6 DNA from the XhoI at bp 15208-13644 (Barker et al., supra (1983)), which also contains the T-DNA boundary sequence (border) implicated in T-DNA transfer. In the plasmid pCGN587, the osc/KAN gene from pCGN552 provides a selectable marker as well as the right border. The left boundary region was first cloned in M13mp9 as a HindIII-SmaI piece (pCGN502) (base pairs 602-2212) and recloned as a KpnI-EcoRI fragment in pCGN565 to provide pCGN580. pCGN565 is a cloning vector based on pUC8-Cm, but containing pUC18 linkers. pCGN580 was linearized with BamHI and used to replace the smaller BglI fragment of pVCK102 (Knauf and Nester, Plasmid (1982) 8:45), creating pCGN585. By replacing the smaller SalI fragment of pCGN585 with the XhoI fragment from pCGN552 containing the ocs/KAN gene, pCGN587 was obtained.

(b) Construction of pCGN739 (Binary Vector)

To obtain the gentamicin resistance marker, the resistance gene was isolated from a 3.1 kb EcoRI-PstI fragment of pPHIJI (Hirsch et al., Plasmid (1984) 12:139-141) and cloned into pUC9 (Vieira et al., Gene (1982) 19:259-268) yielding pCGN549.

The pCGN549 HindIII-BamHI fragment containing the gentamicin resistance gene replaced the HindIII-BglII fragment of pCGN587 (for construction, see 6.1(a), supra) creating pCGN594.

The pCGN594 HindIII-BamHI region which contains an ocs-kanamycin-ocs fragment was replaced with the HindIII-BamHI polylinker region from pUC18 (Yanisch-Perron, Gene (1985) 33:103-119) to make pCGN739.

(c) Construction of 726c (1 ATG-Kanamycin-3' region)

pCGN566 contains the EcoRI-HindIII linker of pUC18 (Yanisch-Perron, ibid) inserted into the EcoRI-HindIII sites of pUC13-Cm (K. Buckley, Ph.D. Thesis,

University of California, San Diego, 1985). The HindIII-BglII fragment of pNW31c-8, 29-1 (Thomashow et al., Cell (1980) 19:729) containing ORF1 and 2 (Barker et al., Plant Mol. Biol. (1984) 2:335-350) was subcloned into the HindIII-BamHI sites of pCGN566 producing pCGN703.

The Sau3A fragment of pCGN703 containing the 3' region of transcript 7 from pTiA6 (corresponding to bases 2396-2920 of pTi15955 (Barker et al., supra (1984)) was subcloned into the BamHI site of pUC18 (Yanisch-Perron et al., supra (1985)) producing pCGN709.

The EcoRI-SmaI polylinker region of pCGN709 was replaced with the EcoRI-SmaI fragment from pCGN587 (see 6.1(a), supra) which contains the kanamycin resistance gene (APH3'II) producing pCGN726.

The EcoRI-SalI fragment of pCGN726 plus the BglII-SalI sites of pUC8-pUC13-cm (chloramphenical resistant, K. Buckley, Ph.D. Thesis, University of California, San Diego, 1985) producing pCGN738. To construct pCGN734, the HindIII-SphI site of M13mp19 (Norrander et al., Gene (1983) 26:101-106). Using an oligonucleotide corresponding to bases 3287 to 3300, DNA synthesis was primed from this template. Following S1 nuclease treatment and HindIII digestion, the resulting fragment was cloned into the HindIII-SmaI site of pUC19 (Yanisch-Perron et al., supra (1985)). The resulting EcoRI to HindIII fragment of pTiA6 (corresponding to bases 3390-4494) into the EcoRI site of pUC8 (Vieira and Messing, supra (1982)) resulting in pCGN734. pCGN726c is derived from pCGN738 by deleting the 900 bp EcoRI-EcoRI fragment.

(d) Construction of pCGN167

pCGN167 is a construct containing a full length CaMV promoter, 1 ATG-kanamycin gene, 3' end and the bacterial Tn903-type kanamycin gene. MI is an EcoRI fragment from pCGN550 (see construction of pCGN587) and was cloned into the EcoRI cloning site in the 1 ATG-

kanamycin gene proximal to the polylinker region of M13mp9. See copending Application Serial No. 920,579, filed October 17, 1986, which disclosure is incorporated herein by reference.

To construct pCGN167, the AluI fragment of CaMV (bp 7144-7735) (Gardner et al., Nucl. Acids Res. (1981) 9:2871-2888) was obtained by digestion with AluI and cloned into the HincII site of M13mp7 (Vieira, Gene (1982) 19:259) to create C614. An EcoRI digest of C614 produced the EcoRI fragment from C614 containing the 35S promoter which was cloned into the EcoRI site of pUC8 (Vieira et al., Gene (1982) 19:259) to produce pCGN146. To trim the promoter region, the BglII site (bp 7670) was treated with BglII and Bal31 and subsequently a BglII linker was attached to the Bal31 treated DNA to produce pCGN147.

pCGN148a containing the promoter region, selectable marker (KAN with 2 ATGs) and 3' region was prepared by digesting pCGN528 (see below) with BglII and inserting the BamHI-BglII promoter fragment from pCGN147. This fragment was cloned into the BglII site of pCGN528 so that the BglII site was proximal to the kanamycin gene of pCGN528.

The shuttle vector used for this construct, pCGN528, was made as follows. pCGN525 was made by digesting a plasmid containing Tn5 which harbors a kanamycin gene (Jorgenson et al., Mol. Gen. (1979) 177:65) with HindIII-BamHI and inserting the HindIII-BamHI fragment containing the kanamycin gene into the HindIII-BamHI sites in the tetracycline gene of pACYC184 (Chang and Cohen, J. Bacteriol. (1978) 134: 1141-1156). pCGN526 was made by inserting the BamHI fragment 19 of pTiA6 (Thomashow et al., Cell (1980) 19:729-739) into the BamHI site of pCGN525. pCGN528 was obtained by deleting the small XhoI fragment from pCGN526 by digesting with XhoI and religating.

pCGN149a was made by cloning the BamHI kanamycin gene fragment from pMB9KanXXI into the BamHI site of pCGN148a. pMB9KanXXI is a pUC4K variant (Vieira and Messing, Gene (1982) 19:259-268) which has the XhoI site missing but contains a functional kanamycin gene from Tn903 to allow for efficient selection in Agrobacterium.

pCGN149a was digested with BglII and SphI. This small BglII-SphI fragment of pCGN149a was replaced with the BamHI-SphI fragment from MI (see below) isolated by digestion with BamHI and SphI. This produces pCGN167.

(e) Construction of pCGN766c (35S promoter-3' region)

The HindIII-BamHI fragment of pCGN167 containing the CaMV-35S promoter, 1 ATG-kanamycin gene and the BamHI fragment 19 of pTiA6 was cloned into the BamHI-HindIII sites of pUC19 (Norrander et al., supra (1985); Yanisch-Perron et al., supra (1985)) creating pCGN976.

The 35S promoter and 3' region from transcript 7 was developed by inserting a 0.7 kb HindIII-EcoRI fragment of pCGN976 (35S promoter) and the 0.5 kb EcoRI-SalI fragment of pCGN709 (transcript 7:3' for construction see supra) into the HindIII-SalI sites of pCGN566 creating pCGN766c.

(f) Final Construction of pCGN783

The 0.7 kb HindIII-EcoRI fragment of pCGN766c (CaMV-35S promoter) was ligated to the 1.5 kb EcoRI-SalI fragment of pCGN726c (1-ATG-KAN-3' region) into the HindIII-SalI sites of pUC119 (J. Vieira, Rutgers University, New Jersey) to produce pCGN778. The 2.2 kb region of pCGN778, HindIII-SalI fragment containing the CaMV 35S promoter (1-ATG-KAN-3' region) replaced the HindIII-SalI polylinker region of pCGN739 to produce pCGN783.

Example 7
Transfer of Genomic Construction to Tomato via Cocultivation

Substantially sterile tomato cotyledon tissue is obtained from seedlings which have been grown at 24°C, with a 16hr/8hr day/night cycle in 100x25 mm petri dishes containing Murashige-Skoog salt medium and 0.8% agar (pH 6.0). Any tomato species may be used, however, here the inbred breeding line was UC82B, available from the Department of Vegetable Crops, University of California, Davis, CA 95616. The cotyledons are cut into three sections and the middle placed onto feeder plates for a 24-hour preincubation. The feeder plates are prepared by pipetting 0.5 ml of a tobacco suspension culture ($\tilde{1}0^6$ cells/ml) onto 0.8% agar medium, containing Murashige minimal organic medium (K.C. Biologicals), 2,4-D (0.1 mg/l), kinetin (1 mg/l), thiamine (0.9 mg/l) and potassium acid phosphate (200 mg/l, pH 5.5). The feeder plates are prepared two days prior to use. A sterile 3 mm filter paper disk containing feeder medium is placed on top of the tobacco cells after the suspension cells are grown for two days.

Following the preincubation period, the middle one third of the cotyledon sections are placed into a liquid MG/L broth culture (1-5 ml) of the A. tumefaciens strain. The binary plasmid pCGN1269 is transferred to A. tumefaciens strain 2760 by conjugation or by transformation selecting for Gentamicin resistance encoded by the plasmid pCGN1269. The cotyledon sections are cocultivated with the bacteria for 48 hrs on the feeder plates and then transferred to regeneration medium containing 500 mg/l carbenicillin and 100 mg/l kanamycin. The regeneration medium is a K.C. Biologicals Murashige-Skoog salts medium with zeatin (2 mg/l) myo-inositol (100 mg/l), sucrose (20 g/l), Nitsch vitamins and containing 0.8% agar (pH 6.0). In 2-3 weeks, shoots are observed to develop. When the shoots are

approximately 1.25 cm, they are excised and transferred to a Murashige and Skoog medium containing carbenicillin (500 mg/l) and kanamycin (50 mg/l) for rooting. Roots develop within 10-12 days.

Shoots which develop and subsequently root on media containing the kanamycin are tested for APH3'II enzyme.

An aminoglycoside phosphotransferase enzyme (APH3'II) assay is conducted on putative transformed tomato plants and shoots. APH3'II confers resistance to kanamycin and neomycin. APH3'II activity is assayed (Reiss et al., Gene (1984) 30:211-218) employing electrophoretic separation of the enzyme from other interfering proteins and detection of its enzymatic activity by in situ phosphorylation of kanamycin. Both kanamycin and [γ-^{32}P] ATP act as substrates and are embedded in an agarose gel which is placed on top of the polyacrylamide gel containing the proteins. After the enzymatic reaction, the phosphorylated kanamycin is transferred to P-81 phosphocellulose ion exchange paper and the radiolabeled kanamycin is finally visualized by autoradiography. The Reiss et al. method is modified in the final washing of the P-81 ion exchange paper by rinsing in 0.1 mg/ml of proteinase K.

Example 8
Construction of Tagged 2A11 Plasmids In Binary Vectors

The complete sequence of the 2A11 genomic DNA cloned into pCGN1273 from the XhoI site (position 1 at the 5' end) to the EcoRI site (position 4654) is shown in Figure 4.

pCGN1267 was constructed by deleting from pCGN1273 a portion of the plasmid polylinker from the EcoRV site to the BamHI site. Two DNA sequences were inserted into pCGN1273 at the unique XbaI site (position 2494). This site is in the 3' non-coding region of the 2A11 genomic clone before the poly A site.

pCGN1273 was tagged with 360 bp (from base number 1 to 360) from the 5' region of the tomato polygalacturonase (PG) cDNA clone, F1 (Sheehy et al., Mol. Gen. Genet. (1987) 208:30-36) at the unique XbaI restriction enzyme site. The tag was inserted in the antisense orientation resulting in plasmid pCGN1271 and in the sense orientation yielding plasmid pCGN1270. Each plasmid was linearized at the unique BglII restriction enzyme site and cloned into the binary vector pCGN783 at the unique BamHI restriction enzyme site.

pCGN1273 was also tagged with a 0.5 kb fragment of DNA (base number 1626 to 2115) from a PG genomic clone (see Figure 5) which spans the 5' end of the intron/exon junction. This fragment was cloned into the XbaI site resulting in plasmid pCGN1215. pCGN1215 was linearized at the unique BglII site and cloned into pCGN783 at the BamHI site resulting in two plasmids, pCGN1219 and pCGN1220, which differ only in the orientation of pCGN1215 within pCGN783.

Three DNA sequences were inserted into pCGN1267 at the unique ClaI sites (position 2402, 2406). These sites are in the 3' non-coding region of the 2A11 genomic clone, 21 bp from the stop codon. The 383 bp XbaI fragment from the PG cDNA clone was cloned into the ClaI site of pCGN1267 after filling in the XbaI and ClaI ends with Klenow and blunt ligation. The fragment in a sense orientation resulted in plasmid pCGN1263 and in the antisense orientation gave pCGN1262. pCGN1263 was linearized at the unique BglII site and cloned into pCGN783 at the BamHI site yielding pCGN1260. pCGN1262 was also linearized at the BglII site and cloned into pCGN783 at the BamHI site resulting in two plasmids, pCGN1255 and pCGN1258, which differ only in the orientation of pCGN1262 in the binary vector pCGN783.

The 0.5 kb fragment of the PG genomic clone spanning the intron/exon junction (supra) was cloned into pCGN1267 at the ClaI site in an antisense direc-

tion yielding plasmid pCGN1225. This plasmid was linearized at the BglII restriction enzyme site and cloned into pCGN783 at the BamHI site producing two plasmids, pCGN1227 and pCGN1228, which differ only in the orientation of pCGN1225 in the binary vector.

The Eco7 fragment (base numbers 5545 to 12,823) (Barker et al., Plant Mol. Biol. (1983) 2:335-350) from the octopine plasmid pTiA6 of A. tumefaciens (Knauf and Nester, Plasmid (1982) 8:45-54) was subcloned into pUC19 at the EcoRI site resulting in plasmid pCGN71. A RsaI digest allowed a fragment of DNA from bases 8487 to 9036 of the Eco7 fragment to be subcloned into the vector m13 BlueScript Minus (Stratagene, Inc.) at the SmaI site resulting in plasmid pCGN1278. This fragment contains the coding region of the genetic locus designated tmr which encodes a dimethylallyl transferase (isopentenyl transferase) (Akiyoshi et al., Proc. Natl. Acad. Sci. USA (1984) 81:5994-5998; Barry et al., ibid (1984) 81:4776-4780). An exonuclease/mung bean treatment (Promega Biotech) produced a deletion on the 5' end of the tmr gene to a point 39 base pairs 5' of the start codon. The tmr gene from pCGN1272 was subcloned into the ClaI site of pCGN1267. The tmr gene in the sense orientation yielded pCGN1261 and in the antisense orientation gave plasmid pCGN1266. pCGN1261 was linearized at the BglII site and cloned into pCGN783 at the BamHI site resulting in plasmid pCGN1254. pCGN1266 was also linearized at the BglII site and subcloned into pCGN783 at the BamHI site yielding two plasmids, pCGN1264 and pCGN1265, which differ only in the orientation of pCGN1266 in pCGN783.

Analysis of Expression in Transgenic Plants

Immature green fruit (approximately 3.2 cm in length) was harvested from two tomato plants cv. UC82B that had been transformed with a disarmed Agrobacterium

strain containing pCGN1264. Transgenic plants are designated 1264-1 and 1264-11. The pericarp from two fruits of each plant was ground to a powder under liquid N_2, total RNA extracted and polyA$^+$ mRNA isolated (as described in Mansson et al., Mol. Gen. Genet. (1985) 200:356-361). Young green leaves were also harvested from each plant and polyA$^+$ mRNA isolated.

Approximately 19 µg of total RNA from fruit, 70 ng of polyA$^+$ mRNA from fruit and 70 ng of polyA$^+$ mRNA from leaves from transformed plants 1264-1 and 1264-11 was run on a 0.7% agarose formaldehyde Northern gel and blotted onto nitrocellulose (Maniatis et al., Molecular Cloning: A Laboratory Manual (1982) Cold Spring Harbor, New York). Also included on the gel as a negative control was approximately 50 ng of polyA$^+$ mRNA from leaf and immature green fruit of a nontransformed UC82B plant.

As a positive control and to help in quantitating mRNA levels, in vitro transcribed RNA from pCGN1272 was synthesized using T3 polymerase (Stratagene, Inc.). Nineteen pg and 1.9 pg of this in vitro synthesized RNA were loaded on the Northern gel.

The probe for the Northern filter was the 1.0 kb tmr insert DNA (a KpnI to SacI fragment) from pCGN1272 isolated by electroelution from an agarose gel (Maniatis, supra (1982)) and labeled by nick translation (Bethesda Research Laboratory kit) using α^{32}P dCTP (Amersham).

The Northern filter was prehybridized at 42°C for 5 hrs in the following solution: 25 ml formamide, 12.5 ml 20X SSC, 2.5 ml 1 M NaP, 5 ml 50X Denhardts, 0.5 ml 10% SDS, 1 ml 250 mM EDTA, 1 ml 10 mg/ml ssDNA and 2 ml H_2O. Then one-fifth volume of 50% dextran sulfate and approximately 2.2X 10^7 cpm of the probe was added and hybridization was for 15 hrs at 42°C.

The Northern filter was washed one time in 2X SSC and 0.1% SDS at 55°C for 20 minutes each wash. The filter was allowed to air dry before being placed with Kodak XAR film and an intensifying screen at -70° for two days.

Northern Results on Transgenic Plants

The nicked tmr probe hybridized with a mRNA species approximately 1.7 kb in length was observed in the total RNA and polyA$^+$ mRNA fruit lanes of the Northern blot. This is the expected length of the reintroduced 2A11 gene (0.7 kb) tagged with the tmr gene (1.0 kb) in the antisense orientation. The level of expression from the reintroduced tagged gene is somewhat lower than the level of expression of the endogenous 2A11 gene. The level of expression of the reintroduced gene in immature green fruit is higher than the expression level in leaf tissue with a small amount of hybridizing mRNA in leaf tissue in these transformants.

Example 9
Screening Genomic Library for Polygalacturonase Genomic Clones

Isolation of a Genomic Clone

An EcoRI partial genomic library established in Charon 4 constructed from DNA of a Lycopersicon esculentum cultivar was screened using a probe from the polygalacturonase cDNA (Sheehy et al., Mol. Gen. Genet. (1987) 208:30-36). A lambda clone containing an approximately 16 kb insert was isolated from the library, of which an internal 2207 bp HindIII to EcoRI was sequenced. The HindIII-EcoRI fragment includes the polygalacturonse promoter region.

Sequence of Genomic Clone

The DNA sequence of the genomic clone was determined by Sanger dideoxy techniques and is as shown in Figure 5. The sequence of the genomic clone bases 1427 to 1748 are homologous to the polygalacturonase cDNA sequence.

The above results demonstrate the ability to identify inducible regulatory sequences in a plant genome, isolate the sequences and manipulate them. In this way, the production of transcription cassettes and expression cassettes can be produced which allow for differentiated cell production of the desired product. Thus, the phenotype of a particular plant part may be modified, without requiring that the regulated product be produced in all tissues, which may result in various adverse effects on the growth, health, and production capabilities of the plant. Particularly, fruit-specific transcription initiation capability is provided for modifying the phenotypic properties of a variety of fruits to enhance properties of interest such as processing, organoleptin properties, storage, yield, or the like.

E. coli strain pCGN1299x7118 was deposited with the American Type Culture Collection (A.T.C.C.), 12301 Parklawn Drive, Rockville, Maryland, 20852 on May 21, 1987 and given Accession No. 67408.

All publications and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A DNA construct comprising in the direction of transcription, a fruit-specific transcriptional initiation region from a gene expressed at or immediately after anthesis or at the breaker stage, said gene remaining expressed at least until the ripe period, joined to a DNA sequence of interest other than the wild-type sequence associated with said initiation region, wherein said DNA sequence of interest is under the transcriptional regulation of said initiation region, and a transcriptional termination region.

2. A DNA construct according to Claim 1, wherein said transcriptional initiation region is from a gene expressed immediately upon anthesis.

3. A DNA construct according to Claim 1, wherein said transcriptional initiation region regulates transcription of a gene encoding a plant storage protein.

4. A DNA construct according to Claim 3, wherein said transcriptional initiation region is the 2A11 region.

5. A DNA construct according to Claim 1, wherein said DNA sequence of interest is a sequence complementary to a native plant transcript.

6. A DNA construct according to Claim 1, wherein said DNA sequence of interest is an open reading frame encoding an amino acid sequence of interest.

7. A DNA construct according to Claim 1, wherein said DNA sequence of interest is a polygalacturonase gene or fragment thereof of at least 12nt in the antisense direction.

8. A DNA construct for integration into a plant genome comprising at least the right T-DNA border joined to a DNA construct according to Claim 1.

9. A DNA construct comprising in the direction of transcription, the fruit-specific transcriptional initiation region of a plant storage protein being active at or immediately after anthesis and remaining active until at least until the ripe period, joined to a DNA sequence other than the wild-type sequence, wherein said sequence comprises a unique restriction site for insertion of a sequence of interest to be under the transcriptional regulation of said initiation region, and a transcriptional termination region.

10. A DNA construct according to Claim 9, wherein said transcriptional initiation region is the 2A11 region.

11. A DNA construct for integration into a plant genome comprising at least the right T-DNA border joined to a DNA construct according to Claim 10.

12. A DNA vector comprising a broad spectrum prokaryotic replication system and a DNA construct according to Claim 1.

13. A DNA vector comprising a broad spectrum prokaryotic replication system and a DNA construct according to Claim 9.

14. A method for specifically modifying the phenotype of fruit substantially distinct from other plant tissue, said method comprising:

transforming a tomato plant cell with a DNA construct under genomic integration conditions, wherein said DNA construct comprises in the direction of transcription, a 2A11 fruit-specific transcriptional initiation region, joined to a DNA polygalacturonase gene sequence, wherein said sequence is oriented in the antisense direction and under the transcriptional regulation of said initiation region, and a transcriptional termination region, whereby said DNA construct becomes integrated into the genome of said plant cell, whereby said antisense sequence is transcribed and inhibits expression of polygalacturonase in fruit;

regenerating a plant from said transformed plant cell; and

growing said plant to produce fruit of the modified phenotype.

15. A method according to Claim 14, wherein said transcription initiation region is the 2A11 region.

16. A plant cell comprising a DNA construct according to Claim 1.

17. A plant cell comprising a DNA construct according to Claim 9.

18. A method for specifically modifying the phenotype of tomato fruit substantially distinct from other plant tissue, said method comprising:

transforming a plant cell with a DNA construct under genomic integration conditions, wherein said DNA construct comprises in the direction of transcription, a fruit-specific transcriptional initiation region being active at or immediately after anthesis, said gene remaining active at least until the ripe period, joined to a DNA sequence other than the wild-type sequence and capable of modifying the phenotype of fruit cells upon

transcription, wherein said sequence is under the transcriptional regulation of said initiation region, and a transcriptional termination region, whereby said DNA construct becomes integrated into the genome of said plant cell;

regenerating a plant from said transformed plant cell; and

growing said plant to produce fruit of the modified phenotype.

19. A plant comprising a DNA construct according to Claim 1.

20. A plant comprising a DNA construct according to Claim 9.

21. Fruit comprising a construct according to Claim 1.

22. Fruit according to Claim 21, wherein said fruit is tomato.

23. Fruit according to Claim 22, wherein said DNA sequence of interest is a polygalacturonase gene or fragment of at least 12nt thereof oriented in the antisense direction and said transcsription initiation region is 2A11.

24. Fruit according to Claim 21, wherein said transcription initiation region is 2A11.

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3H11 TTTTTTTGAGCAAAGGGCAACTCAGATATCCAAAGATGAATCCAACATATA 51

3H11 GCTTACAGCTGGGAGAACATTGTCTAACTCTTCTGAAATTTAAATGTTATC 102

3H11 CAGAATCCTTCATCATAAAATAATATCAAAATGCAAATCTATTTTTTCTAC 153

3H11 TCTTGTCTAGCTTCAACTTTCTTCTTCTGCTCATCAATTAGCAATTAATCC 204
                                  TGCTCATCAATTAGCAATTAATCC

3H11 AAAACCATTATGGCTGCCAAAAATTCAGAGATGAAGTTTGCTATCTTCTTC 255
2A11 AAAACCATTATGGCTGCCAAAAATTCAGAGATGAAGTTTGCTATCTTCTTC
           METAlaAlaLysAsnSerGluMETLysPheAlaIlePhePhe

3H11 GTTGTTCTTTTGACGACCACTTTAGTTGATATGTCTGGAATTTCGAAAATG 306
2A11 GTTGTTCTTTTGACGACCACTTTAGTTGATATGTCTGGAATTTCGAAAATG
     ValValLeuLeuThrThrThrLeuValAspMETSerGlyIleSerLysMET

3H11 CAAGTGATGGCTCTTCGAGACATACCCCCACAAGAAACATTGCTGAAAATG 357
2A11 CAAGTGATGGCTCTTCGAGACATACCCCCACAAGAAACATTGCTGAAAATG
     GlnValMETAlaLeuArgAspIleProProGlnGluThrLeuLeuLysMET

3H11 AAGCTACTTCCCACAAATATTTTGGGACTTTGTAACGAACCTTGCAGCTCA 408
2A11 AAGCTACTTCCCACAAATATTTTGGGACTTTGTAACGAACCTTGCAGCTCA
     LysLeuLeuProThrAsnIleLeuGlyLeuCysAsnGluProCysSerSer

3H11 AACTCTGATTGCATCGGAATTACCCTTTGCCAATTTTGTAAGGAGAAGACG 459
2A11 AACTCTGATTGCATCGGAATTACCCTTTGCCAATTTTGTAAGGAGAAGACG
     AsnSerAspCysIleGlyIleThrLeuCysGlnPheCysLysGluLysThr

3H11 GACCAGTATGGTTTAACATACCGTACATGCAACCTGTTGCCTTGAACAATA 510
2A11 GACCAGTATGGTTTAACATACCGTACATGCAACCTGTTGCCTTGAACAATA
     AspGlnTyrGlyLeuThrTyrArgThrCysAsnLeuLeuPro .
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FIG. 1-1

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3H11 TCAATGATCTATCGATCGATCTATCTATCTATTTATCTGTCTCTGCGCGTA 561
2A11 TCAATGATCTATCGATCGATCTATCTATCTATTTATCTGTCTCTGCGCGTA

3H11 TAGTGTTGTCTGTACCTTTGGTGTGAAGAATATGAATAAAGGGATACATAT 612
2A11 TAGTGTTGTCTGTACCTTTGGTGTGAAGAATATGAATAAAGGGATACATAT

3H11 ATCTAGATATATTCTAGGTAATGTCCTATTGTATTTAAAATTTGTAGCAAT 663
2A11 ATCTAGATATATTCTAGGTAATGTCCTATTGTATTTAAAATTTGTAGCAAT

3H11 GATTGTTTGAATAAAAACATACCATGAGTGAAATAATTATTCCACATTAAT 714
2A11 GATTGTTTGAATAAAAACATACCATGAGTGAAATAATTATTCC

3H11 TCACGTATTTATTTCACTTATGATACGTATTTTTGTTCCTTTCGCGTAAAA 765

3H11 AAAAAAAAA    774
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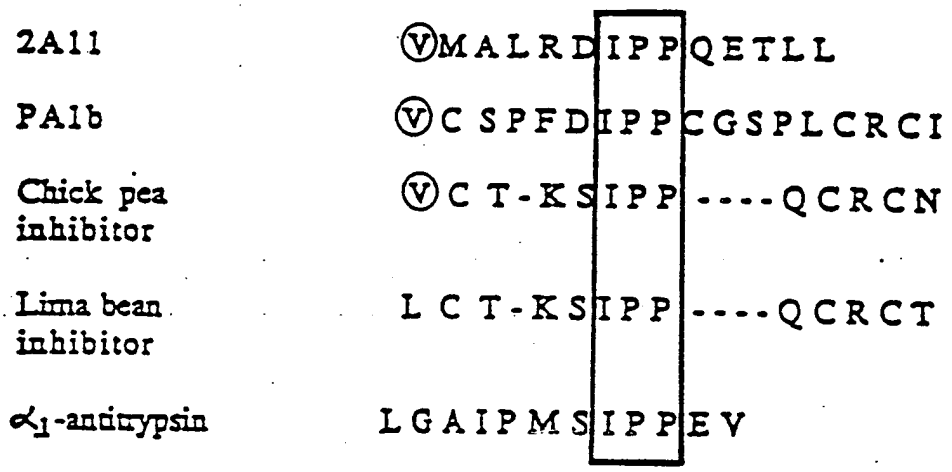
FIG. 1-2

FIG. 2-1


2A11
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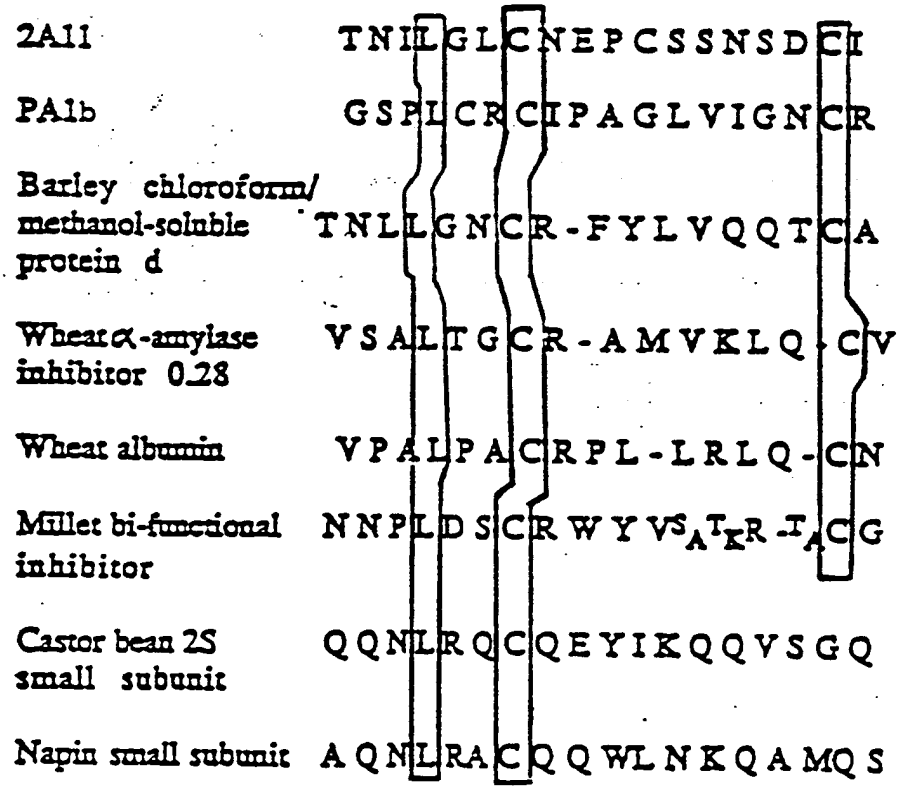
PA1b
ⓋCSPFDIPPCGSPLCRCI
Chick pea inhibitor
ⓋCT-KSIPP----QCRCN
Lima bean inhibitor
LCT-KSIPP----QCRCT
α1-antitrypsin
LGAIPMSIPPEV

FIG. 2-2


2A11
TNILGLCNEPCSSNSDCI
PA1b
GSPLCRCIPAGLVIGNCR
Barley chloroform/ methanol-soluble protein d
TNLLGNCR-FYLVQQTCA
Wheat α-amylase inhibitor 0.28
VSALTGCR-AMVKLQ-CV
Wheat albumin
VPALPACRPL-LRLQ-CN
Millet bi-functional inhibitor
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Castor bean 2S small subunit
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Napin small subunit
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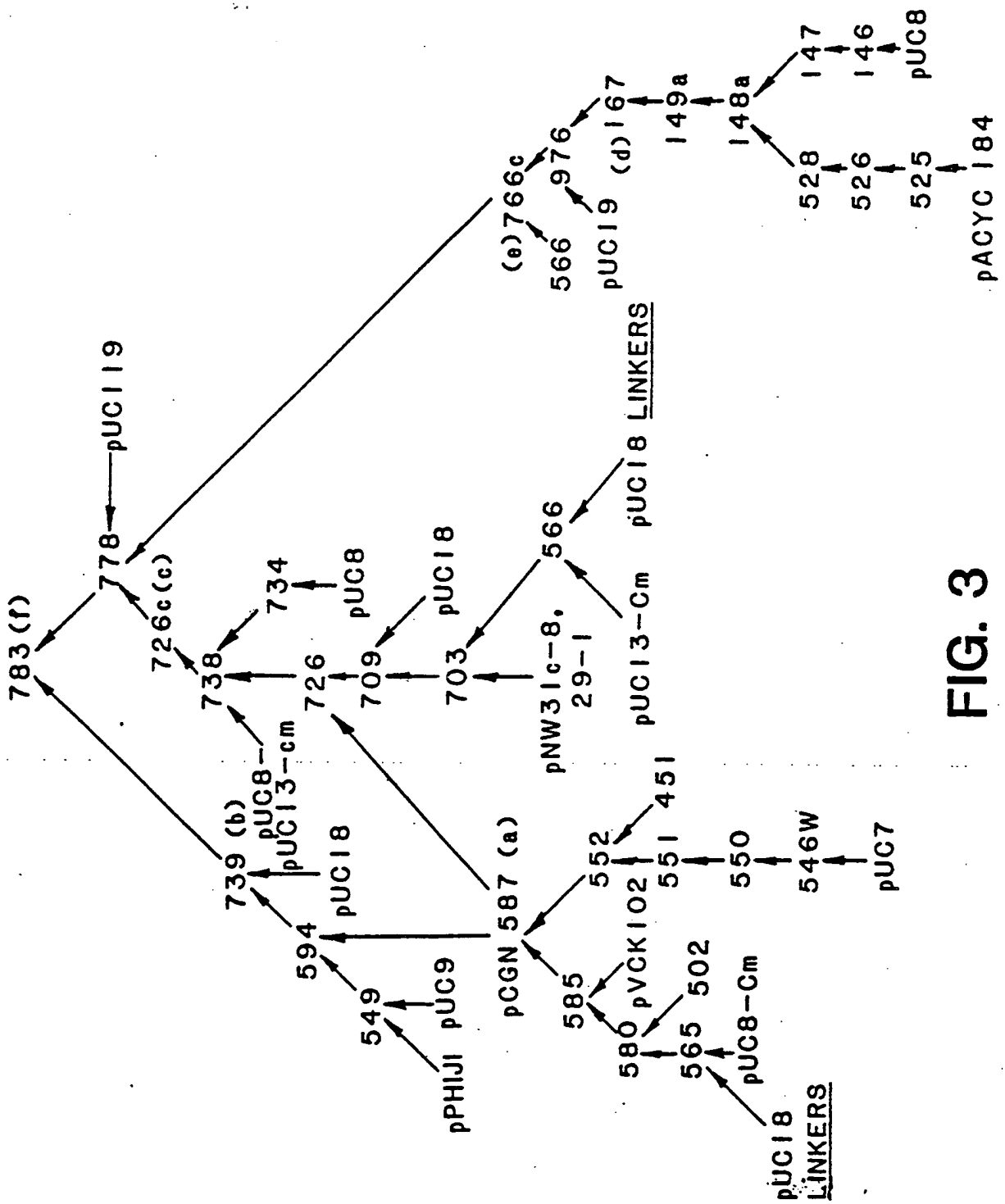
783 (f)
778
pUC119
726c (c)
738
734
pUC8
726
709
pUC18
703
566
pUC18 LINKERS
pNW31c-8, 29-1
pUC13-Cm
739 (b)
pUC8-pUC13-cm
pUC18
594
549
pPHIJI
pUC9
pCGN 587 (a)
552
451
551
550
546W
pUC7
585
pVCK102
580
565
502
pUC8-Cm
pUC18 LINKERS
(e)766c
566
976
pUC19
(d)167
149a
148a
147
146
pUC8
528
526
525
pACYC184

FIG. 3

2A11 GENOMIC

```
        10          20          30          40          50
CTCGAGCCCT TTAAAAAGTA TAGTCAATAT TTACGGTGAC CGTGAATTTC
        60          70          80          90         100
TTAATTATGA TATATAATTT AAAAGAAATC ATGATCACAT TCTACTGATG
       110         120         130         140         150
AGAACATGTG CTAATCAAGG GAAAACATGG ATGTGAAAAA TACTTTTTGT
       160         170         180         190         200
TAAAAGTAAA AAAAAATGTG AAATTTTGTT AGTTATTTAC TACCTATACA
       210         220         230         240         250
TTATTTGAGC ATGTGCAAAC TTTACAAATA CCTAATAGAA GATTTTCACC
       260         270         280         290         300
TGCCTGTATA TATGTAAATT AATTATAATG AACACTCTCA CATAAAATAA
       310         320         330         340         350
TTATCAGTAT ATACATTAAT ACTTGCCCTC CACAATGAAT TAAATAAAAT
       360         370         380         390         400
GTAGAACATG ATCTACACTT CAATAAAACT AAGACCATAA AGAATAATTT
       410         420         430         440         450
CAAAATATAC ACATGTCAAC AATAAATTAT TTGCATATTA TATTAACTTA
       460         470         480         490         500
CTAAACAATC TTTACTTTTG AAATATAAAA ATAATCAAGT TATAAGTCTG
       510         520         530         540         550
CTCAAAGTAA AGCACTTGTT AGACTCATCT GATTTTGAGA AGGTAAGCAA
       560         570         580         590         600
ATTGATGGTG CATAATAGTC ACAAGTAAAA TATAAAATAG ATTTCATTAG
       610         620         630         640         650
TAAAATTGTT TTTTACTTTC TTTATATATA ATTATCAATA TCCTTCAATG
       660         670         680         690         700
GTAGGTTAAT TATATTGTTA ACTTCTTGTT GAATTAAAGC AATAAGACAA
       710         720         730         740         750
GAATATTAAA GATAAAAGAA CAATAAAAAT AGAAAGACTA AGAGATAAGA
       760         770         780         790         800
GTTTTCTTAT TCTTCTTTCA ATAAGTATCA TCAAGTGTAT ACAATATAAA
       810         820         830         840         850
TTTTTGTATT TTTGATCTAT CTATTTATAA TGTTATATAT AAGCATACAA
       860         870         880         890         900
AAGATCAGTC ATAAATATGA CTTTAATCAT GAAAATAATG AAAGAGATTA
       910         920         930         940         950
TGAAGGCGTA AGGTTACTAG AATAATAGTC ATTAAAAAAA GGGGTTATCT
       960         970         980         990        1000
TTATAATTGA ATAATTGATG AAGTAATGGA GATAATTAGT GAGCATAAAT
      1010        1020        1030        1040        1050
TTTTTTAAAA AAATGGACAT TTACACTATA ATATTTTATA ACACTTTCCC
      1060        1070        1080        1090        1100
TTAAACATCT AGGTATAAAT AATGAGTCTT GTCAAAATCT TAGTAGGAAA
```

FIG. 4-1

```
    1110        1120        1130        1140        1150
AATTCTGTGA AATTTTTTTA GTGAAAACAA ATGATATAAA TATCTTGAAT
      1160        1170        1180        1190        1200
ACTCATTATT TGTTGTCTCA TTAAAAATCT TATCTGACCT ATAAAATAAA
      1210        1220        1230        1240        1250
TTATTTGCTC AACTCAAAAT AGTTTTTCAT TCTAAAATTA GTATAATTAT
      1260        1270        1280        1290        1300
TAGTGAATAT TTAATTAACA TAATTGTATA CTAAGGGGCC TATAAATTGG
      1310        1320        1330        1340        1350
ATTCTTCTCA AAGAAAAATA AAATCACCAC ACAACTTTCT TCTTCTGCTC
      1360        1370          1381        1390
ATCAATTAGC AATTAATCCA AAACCATT ATG GCT GCC AAA AAT
                                  MET Ala Ala Lys Asn
   1399        1408        1417        1426
TCA GAG ATG AAG TTT GCT ATC TTC TTC GTT GTT CTT TTG
Ser Glu MET Lys Phe Ala Ile Phe Phe Val Val Leu Leu
1435        1444        1454        1464        1474
ACG ACC ACT TTA GGTTCACAAC ACTTCTCCCT TATTTTGTTT
Thr Thr Thr Leu
      1484        1494        1504        1514        1524
TCTTAATTTC TTGGAAGTCA TATGCATGTG TTTGGTATCA TGGTATATAT
      1534        1544        1554        1564        1574
ATAAAGGAAA ATATTTTTCT TAATTACTGG TTTTCTAATG TTTGGTAGGT
      1584        1594        1604        1614        1624
AATCGGAAAT TATTATGAGA TAATGAACTT GCAAAGTCAT TATTATATAA
      1634        1644        1654        1664        1674
CTTTTTTTTT ATACTTTGAT TTAAGAATTC ATTTTTCTCA TTTTATATAA
      1684        1694        1704        1714        1724
ACTTATTTTT CAACAGAAAA TATTTTTCGA ACTATTCAAA CACACCCTAA
      1734        1744        1754        1764        1774
GACATTACAT ATATATATAT ATACACCCTC CGTTTTATAT TACTTAATGC
      1784        1794        1804        1814        1824
CTATTGAGTT GGCCCACCCT TTAAGAATGA TTCAATTAGA GATATGTTTT
      1834        1844        1854        1864        1874
ACTAAATTAA CCTATGCTTT AAGACTCTAA ATTTGGCTAT TACTATTTTA
      1884        1894        1904        1914        1924
CGTTGTAATT TAATGACAAA CATTTCATAA TGACTATAGT CTGAACTTAA
      1934        1944        1954        1964        1974
TTAGACAGAC GTATCTATAG TTTGCTTACT AATGATTCAT AGCTATATAT
      1984        1994        2004        2014        2024
TTGGAGAGGA GAGAGACAAA CGATATTAAG AAAGGGAGGA GAGAGGCGAG
      2034        2044        2054        2064        2074
GTAAATCTGA AATAGAGAAG AGAAAGGCAA CCAATTTTGA TCATCTATCA
      2084        2094        2104        2114        2124
TACTTTTGAT TATTATTTTT ATTATATGTA CGTTTACATT ACAGTTTTCG
```

FIG. 4-2

```
      2134        2144        2154          2164
AATTCTTACA TTAATCTTAA TCATAATATA TACA GTT GAT ATG
                                          Val Asp MET
   2173        2182        2191        2200
TCT GGA ATT TCG AAA ATG CAA GTG ATG GCT CTT CGA GAC
Ser Gly Ile Ser Lys MET Gln Val MET Ala Leu Arg Asp
2209        2218        2227        2236        2245
ATA CCC CCA CAA GAA ACA TTG CTG AAA ATG AAG CTA CTT
Ile Pro Pro Gln Glu Thr Leu Leu Lys MET Lys Leu Leu
       2254        2263        2272        2281
CCC ACA AAT ATT TTG GGA CTT TGT AAC GAA CCT TGC AGC
Pro Thr Asn Ile Leu Gly Leu Cys Asn Glu Pro Cys Ser
   2290        2299        2308        2317
TCA AAC TCT GAT TGC ATC GGA ATT ACC CTT TGC CAA TTT
Ser Asn Ser Asp Cys Ile Gly Ile Thr Leu Cys Gln Phe
2326        2335        2344        2353        2362
TGT AAG GAG AAG ACG GAC CAG TAT GGT TTA ACA TAC CGT
Cys Lys Glu Lys Thr Asp Gln Tyr Gly Leu Thr Tyr Arg
       2371        2380          2393        2403
ACA TGC AAC CTG TTG CCT TGA ACAATATCAA TGATCTATCG
Thr Cys Asn Leu Leu Pro  .
       2413        2423        2433        2443        2453
ATCGATCTAT CTATCTATTT ATCTGTCTCT GCGCGTATAG TGTTGTCTGT
       2463        2473        2483        2493        2503
ACCTTTGGTG TGAAGAATAT GAATAAAGGG ATACATATAT CTAGATATAT
       2513        2523        2533        2543        2553
TCTAGGTAAT GTCCTATTGT ATTTAAAATT TGTAGCAATG ATTGTTTGAA
       2563        2573        2583        2593        2603
TAAAAACATA CCATGAGTGA AATAATTATT CCACATTAAT TCACGTATTT
       2613        2623        2633        2643        2653
ATTTCACTTA TGATACGTAT TTTTGTTCCT TTCGCGTAGA TTTTTGATCC
       2663        2673        2683        2693        2703
TTTTCCCTTT TGAATATTAA ACATTAAACA CAAATAATGT TTATTAAATT
       2713        2723        2733        2743        2753
AAGTTAATAT TTTTATTTAG CTATTTATAT TTTTATTTGA AATCAAACTT
       2763        2773        2783        2793        2803
GATAAATATT TATAAAGATA ATTAACAAGT AATGTGACAC TAACACCATG
       2813        2823        2833        2843        2853
TAATATTATC TTGTCGTTAT TTATGATAAT ATTTTAAAAT TATAATTTCA
       2863        2873        2883        2893        2903
GTTAAAAAAT TATTAAAAAA ACATACTTTT AAAAAGTGAG TTAGCCTCCG
       2913        2923        2933        2943        2953
CTACCCACAT ACTTATGAAT TGGACTAGTT GTTTTTTGAC CCACAAAAAG
       2963        2973        2983        2993        3003
AATGGGCTAA TTAAACCTGA CCTATCAAAT TTCAGAATCT GCATAGATTA
```

FIG. 4-3

```
      3013        3023        3033        3043        3053
GTCCGAACGA AATGAGTCAG CCCGTATTGA ACAAAATATC AACAAGGACG
      3063        3073        3083        3093        3103
TTATGTAAAG ATGTTTAAGA AGGAAAAAAG ATTTCTAATA CATATGGACT
      3113        3123        3133        3143        3153
TTCAATATCC CAACTTTGTC TGGCGATCTG AACCCTGCTT AGTTTGTTGA
      3163        3173        3183        3193        3203
TCATTAACTT GTCTTGCTAT GTATTTAAGA TTTAAACTTT ATATGTTTAA
      3213        3223        3233        3243        3253
ACTTACAGAA AATACATATA AATCTCTCAA GACTTGGCAA CATAATTTAC
      3263        3273        3283        3293        3303
TTTAGTACTT AAACTACATG AAAATTTAAA TATCCTTTTA ACATCTTTGA
      3313        3323        3333        3343        3353
AGTGAATTAA ATTATCACAA TCCGAGCCTA CACCTTGGAC GTGGCCGGCA
      3363        3373        3383        3393        3403
CTCAAGAACC AGTGCTGGTC CCCAAGCTAA CCCTCATCCT GACTGACTAC
      3413        3423        3433        3443        3453
AAGCGGAAGG CTAACTTAAG TATACAAAAG CTTAAAACTG AATAAAATAA
      3463        3473        3483        3493        3503
ACTTTACAAG GTTTTAACAC AAATGAACAA CTTTGAAGAA AATAATATAT
      3513        3523        3533        3543        3553
TCAACTAGCC ATAAAATAGA CAACTTTAGT CTTTAAAACA TTTAATAAAA
      3563        3573        3583        3593        3603
TAAATGCAAA ATATAGACTC CTTAACTAAA CTGACTATCT ATGGAGCCTC
      3613        3623        3633        3643        3653
TAATTGATAA AGATGGAAGT CGGGACAAGA CCACGACATC CTGACTAAAC
      3663        3673        3683        3693        3703
TGAGAAGTAA ATAAAATCCC CCGGAAAAAA AGGAGCCTCA CCATGGCTAA
      3713        3723        3733        3743        3753
CTCGAACTCG GGGATATATC AATGAAGCTC CTGTTGATGA TCTTGAAGAC
      3763        3773        3783        3793        3803
ATGTCTCTGC ATCATCAAAA AGATGCAGGC CAAATGGCTC AGTACGTAAA
      3813        3823        3833        3843        3853
ATGTACGAGT ATGTAAGGGA AATTCTAAAG TATAACATAA GCTTGATACT
      3863        3873        3883        3893        3903
TGAATAAAAG GAAACATACT TACCTCTTTT CAACTCAACT CAAATTAAGA
      3913        3923        3933        3943        3953
ATAAGATACT CAACTCAAAG ATTAGGTATT CAACGCAAAT ATGGCACTCT
      3963        3973        3983        3993        4003
ACTCAATGAA GTACAAATTA ACTCAGGATA CTCGACTTAA GATACTCAAC
      4013        4023        4033        4043        4053
TCCCGACACT CAACTGAACT CATTTCAATA TAAAGCAGCT TAAAACAAGT
      4063        4073        4083        4093        4103
TCAGTATAAA GTAAAGTTGT TTAAAAACAT GATGTCAACT CTGTGTGTAT
      4113        4123        4133        4143        4153
AATAAGGGAT ACAACATAAC TTTGAAATGT ATATAAAAAT ACAATTAACT
```

FIG. 4-4

```
      4163        4173        4183        4193        4203
GATGTATATA AAAATACATT AATCTATGGG AGATTCTCTA ACCGACAACC
      4213        4223        4233        4243        4253
ATCACTTAAG GGCTAAGATG ATGATATAGC GATCTACCGC ACGCTGCCAT
      4263        4273        4283        4293        4303
CGCATCTTAT ACCCGGCCAA AGGTATAAGA CCTGAACTGC CTAATGAATC
      4313        4323        4333        4343        4353
CACTAATAAA CTGTTAAAAG GAATCATCTA AAAAGTATGA CCCTTTTCTA
      4363        4373        4383        4393        4403
CCCATAGTGG CTAACATGGT TTATGGGGGC TGTGAGTTAT CTGAACTCTC
      4413        4423        4433        4443        4453
CCCCATATCG GTGCTCAATA CTACTCCAAA AAATATACTG CTCTTATGTT
      4463        4473        4483        4493        4503
TAAAAACATA CTGATTCTGT GGTTTGAAAT TATTGCTTAA AGCTTAGATT
      4513        4523        4533        4543        4553
TTTGAAAAGC TCTCTTTTGA AAATCGTAGT TTCCTTTTTC TTCTATTAAA
      4563        4573        4583        4593        4603
GCTAGACATA GGCTATGTAG AACTCTAGCT TACCTTCCTT CTCAAAAGTT
      4613        4623        4633        4643        4653
TGAAAACATT TGCTTAGATT CTTAGGGACT ACTTAGTTCC CTTGTTGGAA
TTC
```

FIG. 4-5

PG GENOMIC

```
        10         20         30         40         50
AAGCTTCTTA AAAAGGCAAA TTGATTAATT TGAAGTCAAA ATAATTAATT

        60         70         80         90        100
ATAACAGTGG TAAAGCACCT TAAGAAACCA TAGTTTGAAA GGTTACCAAT

       110        120        130        140        150
GCGCTATATA TTAATCAACT TGATAATATA AAAAAAATTT CAATTCGAAA

       160        170        180        190        200
AGGGCCTAAA ATATTCTCAA AGTATTCGAA ATGGTACAAA ACTACCATCC

       210        220        230        240        250
GTCCACCTAT TGACTCCAAA ATAAAATTAT TATCCACCTT TGAGTTTAAA

       260        270        280        290        300
ATTGACTACT TATATAACAA TTCTAAATTT AAACTATTTT AATACTTTTA

       310        320        330        340        350
AAAATACATG GCGTTCAAAT ATTTAATATA ATTTAATTTA TGAATATCAT

       360        370        380        390        400
TTATAAACCA ACCAACTACC AACTCATTAA TCATTAAATC CCACCCAAAT

       410        420        430        440        450
TCTACTATCA AAATTGTCCT AAACACTACT AAAACAAGAC GAAATTGTTC

       460        470        480        490        500
GAGTCCGAAT CGAAGCACCA ATCTAATTTA GGTTGAGCCG CATATTTAGG

       510        520        530        540        550
AGGACACTTT CAATAGTATT TTTTTCAAGC ATGAATTTGA AATTTAAGAT

       560        570        580        590        600
TAATGGTAAA GAAGTAGTAC ATCCCGAATT AATTCATGCC TTTTTTAAAT

       610        620        630        640        650
ATAATTATAT AAATATTTAT GATTTGTTTT AAATATTAAA ACTTGAATAT

       660        670        680        690        700
ATTATTTTTT TAAAAATTAT CTATTAAGTA CCATCACATA ATTGAGACGA

       710        720        730        740        750
AGGAATAATT AAGATGAACA TAGTGTTTAA TTAGTAATGG ATGGGTAGTA
```

FIG. 5-1

```
       760         770         780         790         800
AATTTATTTA TAAATTATAT CAATAAGTTA AATTATAACA AATATTTGAG

       810         820         830         840         850
CGCCATGTAT TTTAAAAAAT ATTAAATAGT TTGAATTTAA AACCGTTAGA

       860         870         880         890         900
TAAATGGTCA ATTTTGAACC CAAAAGTGGA TGAGAAGGGT ATTTTAGAGC

       910         920         930         940         950
CAATAGGRGG ATGAGAAGGA TATTTTGAAG CCAATATGTG ATGGATGAAG

       960         970         980         990        1000
GATAATTTTG TATCATTTCT AATACTTTAA AGATATTTTA GGTCATTTTC

      1010        1020        1030        1040        1050
CCTTCTTTAG TTTATAGACT ATAGTGTTAG TTCATCGAAT ATCATCTATT

      1060        1070        1080        1090        1100
ATTTCCGTCT TAAATTATTT TTTATTTTAT AAATTTTTTA AAAATAAATT

      1110        1120        1130        1140        1150
ATTTTTTCCA TTTAACTTTG ATTGTAATTA ATTTTTAAAA ATTACCAACA

      1160        1170        1180        1190        1200
TATAAATAAA ATTAATATTT AACAAAGAAT TGTAACATAA TATTTTTTTA

      1210        1220        1230        1240        1250
ATTATTCAAA ATAAATATTT TTAAACATCA TATAAAAGAA ATACGACAAA

      1260        1270        1280        1290        1300
AAAATTGAGA CGGGAGAAGA CAAGCCAGAC AAAAATGTCC AAGAAACTCT

      1310        1320        1330        1340        1350
TTCGTCTAAA TATCTCTCAT CCAAACTAAT ATAATACCCA TTATAATTAA

      1360        1370        1380        1390        1400
CCATATTGAC CAACTCAAAC CCCTTAAAAT CTATAAATAG ACAAACCCTT

      1410        1420        1430        1440        1450
CCCATACCTC TTATCATAAA AAAAATAATA ATCTTTTTCA ATAGACAAGT

      1460        1470        1480        1490        1500
TTAAAAACCA TACCATATAA CAATATATCA TGGTTATCCA AAGGAATAGT
```

FIG. 5-2

```
      1510        1520        1530        1540        1550
ATTCTCCTTC TCATTATTAT TTTTGCTTCA TCAATTTCAA CTTGTAGAAG

      1560        1570        1580        1590        1600
CAATGTTATT GATGACAATT TATTCAAACA AGTTTATGAT AATATTCTTG

      1610        1620        1630        1640        1650
AACAAGAATT TGCTCATGAT TTTCAAGCTT ATCTTTCTTA TTTGAGCAAA

      1660        1670        1680        1690        1700
AATATTGAAA GCAACAATAA TATTGACAAG GTTGATAAAA ATGGGATTAA

      1710        1720        1730        1740        1750
AGTGATTAAT GTACTTAGCT TTGGAGCTAA GGGTGATGGA AAAACATATG

      1760        1770        1780        1790        1800
ATAATATTGT AAGTATTTAA ATATTGGAAT ATATTTGTGG GGATGAAAAT

      1810        1820        1830        1840        1850
GATAGAGAAT ATAAGAATTA TTTGGAAGGA TGAAAAGTTA TATTTTATAA

      1860        1870        1880        1890        1900
AGTAGAAAAT TATTTTCTCG TTTTTAGTAA TTAAAGGTGA AAAATGAGTT

      1910        1920        1930        1940        1950
TTCTCGTAAG CGAGGAAAGT CATTTTCCAT GGAACTGTAT TTTTTTTTTA

      1960        1970        1980        1990        2000
CTTTTAATAA CGTCATAGTA TTTGCTATAC TCAAGAATAA GACACTATTA

      2010        2020        2030        2040        2050
TTGATGTTTA GTGCTCGAAA AGAAATTGAT AGTAATTTTG CTAATATAAC

      2060        2070        2080        2090        2100
TATCAATTTC TTATATGTAT ATTTTTCAAC CAAAATAACA AAGCGTAATC

      2110        2120        2130        2140        2150
CAATAAGTGG GCCTCTAGAA TAAAGAGTAA GTTCTATTAA TTCTTAACCT

      2160        2170        2180        2190        2200
TATTTAATTT TATGGAAACC TCGACAAAAC GACAATGCTC AACTTATATT

CGAATTC
```

FIG. 5-3

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US88/01811

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (4): C07H 15/12 C12N 15/00 C12N 5/00 A01H 1/04

U.S. CL: 536/27 435/172.3 435/320 435/240.4 800/1

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	435/172.3, 240.4, 320 536/27 800/1

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X Y	Plant Physiology, Volume 83, issued April 1987, (Rockville, Maryland, USA), Boston et al., "Expression from heterologous promoters in electroporated carrot protoplasts", pages 742-746, see pages 742-743 in particular.	1-3,6,9, 12,13, 16,17 8,14, 18-24
Y	Molecular and General Genetics, Volume 200, issued August 1985, (Heidelburg, Germany), Mansson et al., "Characterization of fruit specific cDNAs from tomato", pages 356-361, see pages 356,358 and 360 in particular.	1-6,10, 14,15, 18-24

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
23 JULY 1988	07 SEP 1988
International Searching Authority	**Signature of Authorized Officer**
ISA/US	DAVID T. FOX David T. Fox

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	Bio/Technology, Volume 3, issued March 1985, (New York, New York, USA), Facciotti et al., "Light-inducible expression of a chimeric gene in soybean tissue transformed with Agrobacterium," pages 241-246, see page 241 in particular.	1-3,8,9, 11,14, 18-24
Y,P	Molecular and General Genetics, Volume 208, issued June 1987, (Heidelburg, Germany), Sheehy et al., "Molecular characterization of tomato fruit polygalacturonase", pages 30-36, see pages 30 and 33 in particular.	7,14,18, 23
Y	Proceedings of the National Academy of Sciences USA, Volume 83, issued September 1986, (Washington, D.C., USA), Della Penna et al., "Molecular cloning of tomato fruit polygalacturonase: analysis of polygalacturonase mRNA levels during ripening," pages 6420-6424, see page 6422 in particular.	7,14,18, 23
Y	Nucleic Acids Research, Volume 14, issued November 1986, (Oxford, England), Grierson et al., "Sequencing and identification of a cDNA clone for tomato polygalacturonase," pages 8595-8603, see pages 8598-8599 in particular.	7,14,18, 23
Y	Proceedings of the National Academy of Sciences USA, Volume 83, issued August 1986, (Washington, D.C., USA), Ecker et al., "Inhibition of gene expression in plant cells by expression of antisense RNA," pages 5372-5376, see page 5373 in particular.	5,7,14, 23

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y	Proceedings of the National Academy of Sciences USA, Volume 82, issued May 1985, (Washington, D.C., U.S.A.), Sengupta-Gopalan et al., "Developmentally regulated expression of the bean beta-phaseolin gene in tobacco seed," pages 3320-3324, see page 3321 in particular.	1-3,8,9, 11,14, 18-24

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . because they relate to subject matter [2] not required to be searched by this Authority, namely:

2. ☐ Claim numbers . because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [3], specifically:

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.